ON ABSORPTION FROM AND SECRETION INTO THE SEROUS CAVITIES. BY ERNEST H. STARLING, M.D., M.R.C.P. AND ALFRED H. TUBBY, M.S. Senior Demonstrator of Physiology at Guy's Hospital.

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IT has long been known that solid particles are absorbed from the serous cavities, and that some time after their introduction they are found in the lymphatics lying under the serous membranes; and Recklinghausen's observations showed that the stomata were the channels by which this transference took place. The question as to the mode of absorption of fluids and substances in solution is still an open one. Although it has been answered in various ways by different authorities, yet the nature of these answers was more determined by theoretical considerations than by actual experiment.

The question then which we had to decide was, Does the absorption of fluids and dissolved substances from the serous cavities take place entirely by way of the stomata and lymphatics, or is it also effected directly by means of the blood vessels lying in the serous membrane? The allied question as to the absorption of dissolved substances from the spaces of connective tissues has often been the subject of investigations, which have resulted in the conclusion that many fluid substances diffuse through the wall of larger and smaller vessels and so get from the tissues into the blood.

The experiments of Prochaska, Magendie and others, which were directed to solve the above question, proved that the absorbed substances really reached the blood by passing through the blood vessel walls. In these experiments, substances such as sulphocyanates, or colouring matters were used, since they could be easily detected after absorption. In order to be certain that the absorption took place only by means of the blood vessels several methods were used. In one experiment Magendie laid bare a long piece of a large vessel, cut it through in two places, and restored the connexion by means of two tubes. Under the isolated piece of vessel was then placed a plate or metal trough containing the solution which was to be absorbed. In other experiments all the lymphatics coming from the part were ligatured, or all the tissues of a limb cut through with the exception of a main artery and vein. Under these circumstances, injection of strychnine for example under the skin of the limb was followed by the usual physiological results, showing that the poison must have been absorbed by means of the blood vessels. Heidenhain has objected to these experiments of Magendie's that proper care was not taken to ensure the absence of an increased tension produced by the forcible injection of fluid under the skin. The subject has been recently reinvestigated with proper precautions by Ascher, under Kühne's superintendence. This worker has entirely confirmed Magendie's conclusions. A soluble substance, such as potassium iodide, allowed to drop on to an open wound, is absorbed directly into the blood, without having to take a devious route through the lymphatic channels and ductus thoracicus.

Arguing from analogy, one would expect the same thing to hold good for the serous cavities. Magendie found that, after introduction of poison into the pleural cavity, death took place earlier if the volume of circulating blood had been diminished, and later, if it had been increased by injection of fluid into the circulation. This dependence of the absorption of the poison on the quantity of the circulating fluid seemed to point to the passage being directly into the fluid, and Ludwig, in his text-book, certainly looks upon the absorption from serous cavities as conforming to the same laws as absorption from connective tissue spaces.

Dybkowsky¹ distinguishes two modes of absorption from the pleural cavity. In the first mode, fluids with suspended particles are pumped through the stomata into the lymphatics by the respiratory movements. The second consists in an interchange between the blood in the blood vessels and the fluid in the pleural cavity. This occurs by diffusion and filtration over the whole surface of both visceral and parietal pleuræ, and cannot, Dybkowsky says, be observed by itself. He points out that, on injection of concentrated solutions of sugar

into the pleural cavity, the sugar disappears very rapidly from the

¹ Dybkowsky. Ludwig's Arbeiten, 1866, p. 191.

pleural fluid and appears in the urine. On the other hand, substances such as proteid diffuse from the blood into the pleural fluid.

In spite of these experiments, Recklinghausen's observations on the passage of fluids, such as milk, through the stomata of the diaphragm, have attracted so much attention that the second mode of absorption from the serous cavities has sometimes been quite overlooked. Physiologists are inclined to ascribe the quick absorption of fluids and dissolved substances from these cavities to the admirable arrangement of the absorbents or lymphatic apparatus with which they are provided, and we ourselves regarded it as entirely open to question whether any absorption took place with the intermediation of the blood vessels at all.

Method of experiment.

The solution of this question presents no experimental difficulties. The animals, which were dogs varying from 4 to 8 kilos in weight, were anæsthetised with morphia and A.C.E. mixture. The thoracic duct was then exposed in the usual way and a cannula inserted, and the lymph collected in graduated cylinders. In several cases the right thoracic duct was ligatured at the beginning of the experiment. An opening was then made into the left pleural cavity and a Y-shaped cannula inserted. One limb of the Y, which projected into the pleural cavity, was securely fastened by a ligature round the skin of the wound. The second limb was connected by a rubber tube (clamped) with a burette, and the third limb was provided with a rubber tube which fitted the nozzle of a brass syringe. The air which had entered the pleural cavity during the operation was sucked out by means of a syringe, so as to restore the negative pressure in the cavity, as was shown by the quiet regular breathing of the animal during the experiment. A cannula was then tied into the ureter, or in most cases into the bladder, either through an opening in the abdominal wall or through an opening in the urethra, which was exposed by splitting the symphysis pubis.

Our object of course was to see whether soluble substances injected into the pleural cavity appeared first in the thoracic duct or in the urine. If they appeared in the urine at all after ligature of both thoracic ducts they must have passed through the walls of the blood vessels, and presumably through those of the pleura. A further clue as to the channels of absorption would be furnished by the amount of flow from the thoracic duct. Thus if 20 c.c. of fluid disappeared in the course of the experiment from the pleural cavity, a considerable increase of flow from the thoracic duct would be expected if the absorption took place in this way. It will be seen from our experiments that the lymphatic resorption is a very slow process, and is indeed so small that it may be disregarded in an experiment lasting from 3 to 4 hours.

The preparations being complete, a measured amount of normal saline fluid holding in solution a substance whose absorption we wished to investigate, was run into the pleural cavity from the burette. The animal was kept then from 2 to 5 hours, during which time the lymph and urine were collected. At the end of this time the dog was killed by blowing air into its veins, and a post mortem examination made at once, the first step in the examination being the collection and measurement of the residual fluid in the pleural cavities. This fluid was afterwards in most cases examined with the view of determining the changes it had undergone during its sojourn in the pleural cavity.

We have made twelve experiments on the absorption of colouring matters (indigo-carmine and methylene blue) from the serous cavities. In nine experiments fluid was put into the pleural cavity and in three into the peritoneal. Of two of these we give the protocols (Exp. I. II.). In all cases except one the urine became coloured before the lymph. In this one experiment no urine was secreted at all. The lymph became slightly tinged with blue half-an-hour after the injection of fluid into the pleura. In the others, 5-20 minutes after the injection of the coloured fluid into the serous cavities the urine became tinged with blue, when indigo-carmine was used, or green, when methylene blue was the substance employed. The urine, on shaking with air, rapidly deepened in tint, until the colour was as intense as that of the fluid injected. After a further lapse of time, varying from ten minutes to four hours, the lymph flowing from the thoracic duct also became slightly tinged, but the colour never deepened, on shaking with air, beyond a very light blue or green. The flow of lymph was not increased. The exact period at which the colour appeared in the urine and lymph respectively seemed to be dependent on the rapidity at which these two fluids were being secreted. Thus in one case where the flow of lymph was very slow, the urine became green within twenty minutes, while no colour appeared in the lymph until the lapse of $5\frac{1}{2}$ hours. In other cases, where the flow of lymph was more abundant than that of urine, the colour appeared in the lymph ten minutes after the urine became green. It will be seen from Exp. II. that the same thing holds good for the peritoneum as for the pleura.

It might be argued from these experiments that the absorption of the colouring matter from the pleural or peritoneal cavity took place by means of the blood vessels and by means of the lymphatics, the former process however being the quicker of the two. It is very doubtful however whether the slight colouration of the lymph which was observed in these experiments is occasioned at all by lymphatic absorption. If methylene blue or indigo-carmine be injected into the blood stream, the lymph flowing from the thoracic duct within half a minute becomes coloured. Now in these cases the colouring matter must have been present in the blood in order to have been excreted by the kidneys, and the colour of the lymph may be caused by a passage of coloured lymph from the blood vessels, not by any direct absorption of the blue from the serous cavities. In Exp. I. and II., specimens of blood were drawn from the femoral artery during the course of the experiment. These were allowed to clot and centrifuged. The serum of all specimens of blood taken during the secretion of coloured urine was tinged with blue. In Exp. II., the tint of the serum was nearly the same as that of the lymph obtained during the same period; and in Exp. I., there was very little difference between the specimens of serum and the lymph secreted at the same time.

So far then our experiments prove conclusively that dissolved colouring matters are taken up directly by the blood vessels from the serous cavities, but afford no evidence of any absorption by way of the lymphatics. That such absorption does take place is shown by the staining of a gland in the anterior mediastinum in Exp. II., and is also proved incontestably by the experiments of all authors who have worked on lymphatic absorption. We have only to consult Dybkowsky's experiments however, to see that this mode of absorption is an extremely slow one, at any rate under normal conditions of respiration.

At the end of the experiments, which lasted from two to six hours, the dog was killed and the fluid withdrawn from the chest. This was found to have undergone certain changes; 10 to $30^{\circ}/_{\circ}$ of the fluid had disappeared; the blue colour was less intense, and the fluid contained a variable amount of coagulable proteid. When put aside, a delicate clot formed in many cases in the fluid. We see here that an interchange had taken place between the fluid in the pleura and the blood in the surrounding blood vessels, each taking from the other that constituent which it did not possess. We see in fact that the same relationship exists between the blood and the fluid in the serous cavities, as between the blood and the lymph in the lymph spaces in the various tissues of the body, and that, as pointed out by Heidenhain, the lymph flow from a part affords no conclusive criterion of the chemical changes going on in that part.

The Injection of Lymphagogues.

At the close of his paper on lymph formation Heidenhain says, 'Pathology, even to-day, gives no clear answer to the simple question,why does the sting of a bee cause local œdema? Possibly my experiments may open the way to a clearer understanding of the matter.' The discovery by Heidenhain of the class of bodies which he terms lymphagogues, naturally suggests the thought that the local cedema produced by various lesions may be due not to a pathological affection of the blood vessels, but may be a physiological reaction of the capillary endothelium, a real secretion of lymph, evoked by the presence of lymphagogue substances, either arising in the tissues themselves or introduced from without, which excite the secretory activity of the cells. There would be a difference however between the conditions in these cases and in Heidenhain's experiments. In the latter the lymphagogue substance is present within in the blood vessels. In the case of the sting of a bee the presumed lymphagogue would be present in the tissue spaces and therefore outside the blood vessels. The question then arises whether any of the first class of lymphagogues will exert the same influence on lymph production when applied to the outside of the vessels. The analogy between the pleural cavity and the other lymph spaces of the body enables us to put this question to the test of experiment. If these lymphagogues act as such when applied to the outside of any vessel, we might expect their presence in the pleural cavity to cause an increased secretion of fluid into that cavity.

This is not however the case. We have experimented with three substances, "peptone" (Grübler's), decoction of mussels, and tissue fibrinogen. In no case was the fluid in the pleura increased. The absorption of fluid took place as in our experiments with methylene blue, and there was at the same time an interchange between blood and pleural fluid.

Thus when a solution $(5^{\circ}/_{o})$ of peptone in normal saline was injected, peptone appeared in the urine after the lapse of two hours.

PH. XVI.

The dog was killed at the end of three hours, when the same amount of fluid was recovered from the pleural cavity as had been injected. It contained however only $1\cdot3^{\circ}/_{\circ}$ of peptone, and about $1\cdot4^{\circ}/_{\circ}$ of coagulable proteid. A delicate clot was formed in the fluid on standing, showing that the presence of this large amount of peptone had no inhibitory influence on the coagulability of the fluid. (See Exp. IV.)

In Exp. V. a decoction of mussels was injected. (The efficacy of this extract as a lymphagogue had been proved by previous experiments.) In this case the fluid in the pleural cavity had diminished in $2\frac{1}{2}$ hours from 50 c.cm. to 40 c.cm.

The result was the same when a solution of tissue fibrinogen was introduced.

Our experiments then afford us no grounds for looking on a locally increased production of lymph as due to the presence of lymphagogue substances in the lymph spaces of the part affected, although they are not conclusive against it.

It must be remembered however that these substances such as mussel extract and peptone have been only proved to have an influence on the flow from the thoracic duct, and their influence on the lymph flow from other than the abdominal viscera has not yet been investigated. It is quite possible that their influence is confined to the vessels of the abdominal viscera¹.

Our next step was to investigate the action of lymphagogues of the second class when injected into the pleural cavity. We give the protocols of three experiments (Exp. VI., VII. and VIII.).

It will be seen from these that injection of concentrated solutions of sugar or salt into the pleural cavity causes a copious flow of fluid into the cavity.

Thus in Exp. VI. at 11.55 a.m. 50 c.cm. of a $10^{\circ}/_{\circ}$ solution of dextrose were put into the pleural cavity. The dog was killed at 1.40, when 96 c.cm. of fluid were recovered.

In Exp. VII. 50 c.cm. of a $10 \,^{\circ}/_{o}$ solution of dextrose in normal saline were run into the pleura. Two hours later the pleural cavity contained 88 c.cm. This fluid only contained $0.5 \,^{\circ}/_{o}$ of sugar, and a small amount of proteid.

In Exp. VIII. 50 c.cm. of a $10^{\circ}/_{\circ}$ solution of NaCl were run into the pleural cavity. At the end of $3\frac{1}{2}$ hours the dog was killed and 170 c.cm. of fluid taken out of the chest.

¹ Cp. This Journal, Vol. xiv. p. 150. 1893.

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We have spoken of these substances as lymphagogues, since it was their action on the lymph flow that led us to study their effect when introduced into the pleural cavity. But it is evident that we have here to do, not with an active secretion of fluid from the blood vessels, but with a more physical process—a diffusion of fluid from the blood into the pleural fluid determined by the high "tonicity" or osmotic pressure of the latter. Thus NaCl has an osmotic pressure which is more than double that of an equal weight of grape sugar, so that a 10 % solution of NaCl would be isotonic with a 27 % solution of dextrose.

Hence we get a much larger effusion of fluid into the pleural cavity after injection of a $10^{\circ}/_{\circ}$ solution of NaCl, than after injection of a $10^{\circ}/_{\circ}$ solution of dextrose.

It is instructive to compare with these experiments the results of injecting hypotonic solutions, such as $75 \,^{\circ}/_{\circ}$ saline (which, as Hamburger has pointed out, is only normal for the frog) and distilled water. Details of two such experiments are given in Protocols III. and IX. In Exp. III. 40 c.cm. of $75 \,^{\circ}/_{\circ}$ NaCl solution were injected at 11 55. $4\frac{1}{2}$ hours later, when the dog was killed, 25 c.cm. of fluid were taken out of the pleura.

In Exp. IX. 50 c.cm. distilled water were injected. $1\frac{1}{2}$ hours later on 30 c.cm. were found, showing that 20 c.cm. had been absorbed in this short time.

In both these cases in which the injected fluids were hypotonic to the blood plasma, there was a movement of fluid from the pleural cavity into the blood vessels.

So far then the absorption of fluid from or secretion into the pleura seems to be determined simply by the osmotic pressure of the fluid already in the pleura. We thought therefore that when any fluid was injected into the pleural cavity there would be a transference of fluid from blood vessels to pleural fluid or vice versâ, until the osmotic pressures on both sides of the endothelium were equal. During this time an exchange of the solids of the blood and pleural fluid would also take place, and this would continue after the osmotic pressures were equalised, until the chemical constitution of the two fluids were also equalised.

After the establishment of osmotic equilibrium it would seem that the absorption of fluid from the pleural cavity is extremely slow, so that it might perhaps be effected by the lymphatics alone.

The absorption of normal saline for instance proceeds at first rapidly. After 2 hours however, when the fluid contains coagulable proteids

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and is alkaline in reaction, the absorption goes on much more slowly, so that whereas 20 cc. of fluid may be absorbed in the first two hours, some of the fluid may, as Dybkowsky showed, be still unabsorbed 24 hours later.

This view is identical with that put forward by Johannes Müller¹, on, so far as we know, a *priori* grounds.

If this hypothesis be correct, then on injecting a fluid having the same composition as the circulating blood, we ought to find that the absorption is very slow, and that the composition of the fluid remains unaltered.

In Exp. X. 50 c.cm. of defibrinated blood, taken from the dog's femoral artery, were injected into the pleura at 12.45. At 4.5 the dog was killed and 51 c.cm. of fluid recovered. The fluid injected contained $19.8 \,^{\circ}/_{\circ}$ solids. The fluid obtained at the end of the experiment contained $18.7 \,^{\circ}/_{\circ}$ solids, so that there had been an exsudation of water from the blood vessels into the pleural cavity.

During this time however the circulating blood must have also become less concentrated in consequence of the withdrawal of the 100 c.cm. blood. In another similar experiment therefore we determined the total solids in the defibrinated blood taken from the artery at the beginning and end of the experiment, and also in the defibrinated blood taken from the pleural cavity.

Oct. 16, 1893. Dog about 6 kilos.

Bled to 100 c.cm. at 2.10.

At 2.25 ran in 50 c.cm. of defibrinated blood into pleural cavity. This contained 19.95 $^{\rm o}/_{\rm o}$ solids.

At 4.25 dog killed by bleeding.

45 c.cm. of fluid recovered from pleural cavity. This contained 19.79 $^{\rm o}/_{\rm o}$ solids.

The first portion of blood obtained at 4.25 was defibrinated. This contained $18.67 \, {}^{\circ}/_{\circ}$ solids.

So that in this case the diminution in total solids of the blood seems to explain the diminution in total solids of the blood injected into the pleura.

With injection of serum we obtained similar results.

¹ Cp. Johannes Müller, *Elements of Physiology*, Baly's translation. Vol. 1. p. 248, 1838.

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Oct. 19, 1893. Dog about 5 kilos.

At 11.10 50 c.cm. of blood serum (from another dog) run into pleural cavity. This contained $8.4 \, {}^{\circ}/_{o}$ solids.

. Dog bled from femoral artery at 1.20. This blood centrifuged. Serum contained $7.2 \, {}^{\circ}/_{\circ}$ solids.

At 1.25 dog killed.

59 c.cm. of fluid recovered from chest. This contained 7.14 % solids.

Here again we obtained equilibrium between the fluids on the two sides of the pleural endothelium. It seems then that the absorption of fluid from the chest by means of the blood vessels and secretion of fluid into the chest are determined by the chemical characters of the fluid already in the chest.

It is a natural conclusion from these experiments to say that these two processes of absorption and secretion are osmotic, and dependent on the varying osmotic pressures on the two sides of the pleural wall. This however is not the case. We have already shown that $75 \,^{\circ}/_{\circ}$ saline is absorbed at first fairly rapidly from the pleural cavity. We thought that if we used a saline fluid that was normal for mammals, viz. a solution of NaCl containing $92 \,^{\circ}/_{\circ}$ salt, that the *amount* of fluid would remain stationary, and that we should find merely an alteration in its composition at the end of a few hours. We found however that saline of this composition was absorbed as rapidly as $75 \,^{\circ}/_{\circ}$ saline.

Thus on Oct. 9, 1893, we injected 50 c.cm. $0.92^{\circ}/_{\circ}$ saline into the pleura at 11.0 a.m. At 2.30 the dog was killed, and only 26 c.cm. could be recovered.

On Oct. 13 we injected 50 c.cm. $1\cdot 2^{\circ}/_{\circ}$ saline into the left pleura of a dog at 2.5 p.m. The dog was killed at 4.5, when only 42 c.cm. of fluid could be recovered.

We then increased the amount of salt still further.

Dog about 6 kilos. At 10.45, injected 50 c.cm. $1.5 \,^{\circ}/_{\circ}$ NaCl solution into pleural cavity. Dog killed at 12.45; 67 c.cm. of fluid recovered from pleural cavities. This only contained $0.64 \,^{\circ}/_{\circ}$ NaCl.

In another experiment 50 c.cm. of $1 \, {}^{\circ}_{\circ}$ NaCl solution were injected. 2 hours later only 43 c.cm. could be recovered.

On Nov. 8 we tried again the effect of $1.5 \,^{\circ}/_{\circ}$ saline. 50 c.cm. of $1.5 \,^{\circ}/_{\circ}$ saline injected into pleura at 1.30. At 2.30 dog killed and 55 c.cm. fluid recovered. This contained $0.58 \,^{\circ}/_{\circ}$ NaCl.

It is evident from these experiments that although a great excess of osmotic pressure in the pleural cavity can determine a flow of fluid from the blood into the cavity, yet if the difference between the osmotic pressures in the pleural fluid and blood is not excessive, fluid will be taken up by the blood. The cells between the blood and the pleural fluid seem to exert a pull on the latter; in fact there is an *active* absorption going on. The cells must perform a considerable amount of work in this absorption, but the work they can do has a limit. If this limit be passed then the passage of fluid follows the osmotic difference. So that we must look upon the living cells as being actively concerned in the absorption of a fluid such as $1^{\circ}/_{0}$ saline.

On the other hand, there can be no doubt that this absorption is conditioned (whether physically or physiologically we cannot at present say) by the chemical constitution of the pleural fluid. If this latter has not only, a 'normal' osmotic pressure, but also a nearly 'normal' composition, then the flow of fluid takes place so as to make its composition the same as that of the circulating plasma. Whether with an absolutely normal fluid in the pleural cavity any absorption by way of the blood vessels takes place at all, we cannot say. At any rate absorption under these circumstances takes place extremely slowly, so that we cannot exclude the possibility that the whole of it takes place by the lymphatics, as Müller suggested.

Two more experiments may be quoted as bearing out these conclusions.

Nov. 2, 1893. Dog about 10 kilos. At 2.40, 70 c.cm. of a mixture of equal parts of dog's serum and $95 \,^{\circ}/_{\circ}$ NaCl solution injected into the pleura. At 4.40 dog killed; 67 c.cm. of fluid recovered.

The injected fluid contained $4.3 \,{}^{\circ}/_{\circ}$ total solids. The fluid taken out of the chest contained $4.5 \,{}^{\circ}/_{\circ}$ total solids. The blood serum (from femoral artery) of the dog contained 7.6 ${}^{\circ}/_{\circ}$ solids.

Nov. 10. Dog 7 kilos. At 9.35 a.m. injected 50 c.cm. of $1.5 \, {}^{o}/_{o}$ NaCl solution into pleura.

At 6 p.m. dog killed; 52 c.cm. fluid obtained from chest. This contained $\cdot71 ^{\circ}/_{\circ}$ NaCl and a considerable amount of coagulable proteid.

Although our experiments thus point to the fact that the absorption of fluids from the serous cavities is not a mere question of osmosis, they do not give a clear answer as to the exact mode of absorption under all circumstances. For instance normal blood serum is absorbed with extreme slowness. On the other hand a solution which, according to Hamburger, has an osmotic pressure equal to normal serum, is absorbed rapidly. What is the factor in determining the difference in the rate of absorption of these two fluids it is difficult to say. Perhaps it is that the endothelial cells are stimulated to active absorption by the presence of any abnormal fluid in the pleural cavity. So soon as the fluid, in consequence of interchanges between it and the blood plasma, has become a normal pleuritic fluid, then this stimulus is removed, and absorption then proceeds extremely slowly, perhaps only by way of the lymphatics. We are therefore still in ignorance as to the most important question in the whole subject, namely the manner in which an ordinary pleuritic effusion is absorbed. We hope however to investigate this subject further.¹

Conclusions.

I. Our experiments show that colouring matters in solution placed in serous cavities are absorbed directly and rapidly by the blood vessels; and this is accompanied by an interchange between the fluid in the cavities and the blood in the vessels.

II. The presence of blood lymphagogues in the pleural cavity does not cause an increased secretion of lymph into that cavity, but an interchange between the blood and pleural fluid goes on as with injection of indifferent substances.

III. Absorption from or secretion into the pleural cavity is not a mere question of osmosis. There is evidence to show that with certain solutions an *active* absorption from the cavity may take place, whether by the blood vessels or pleural endothelium we are at present unable to determine.

PROTOCOLS.

Anæsthetics (cp. text) were in all cases used.

EXPERIMENT I.

June 6, 1893. Bitch about 6 kilos. Cannulae in thoracic duct and bladder, and in left femoral artery for bleeding. Y cannula in left pleura. Right lymphatic duct ligatured.

TimeLymphUrine3.30-40' = .4 c.cm.Colourless5.8 c.cm.At 3.40, 50 c.cm. of $\frac{1}{2}$ °/₀ sol. of Indigo carmine (Grübler) in normal saline run into pleura.

¹ It is worth noting that in a recent paper Kolossow denies the existence of stomata, or of any direct communication between pleuroperitoneal cavities and lymphatics.

Time	\mathbf{Lymph}	Urine			
3.40—50'	= ·4 c.cm.	Colourless	5 c.cm.	Blue	at 3.47
3.50-4.20	= 1.4 c.cm.	"	12 c.cm.	Dar	k blue
4.20-40'	= 1.4 c.cm.	Slight tinge of blue	7 c.cm.	Very	dark blue
4.40-5.20	= 7 c.cm.	Light blue	5 c.cm.	,,	,,
(A clot removed from cannula at 5.10.)					
5.20-5.55	= 8.4 c.cm.	Light blue	3.8 c.cm.	,,	,,

Dog killed at 6 p.m. At 3.56, 4.20, 4.40, and 5.15 specimens of blood were taken from the femoral artery. These were allowed to clot and then centrifuged. The serum in all four specimens was blue, no. 1 being only faintly tinged, and the tint gradually increasing in depth to no. 4. In no. 4 the colour was not so intense as in the corresponding specimen of lymph.

In the pleural cavities were found 38 c.cm. on left side,

" " " " " " " 3 c.cm. on right side. The fluid which was blue contained a small amount of proteid.

EXPERIMENT II.

June 5, 1893. Bitch about 5 kilos. Cannula in thoracic duct, and cannula in bladder (symphysis split).

Time	\mathbf{Lymph}		Urine		
$2.40_{}50'$	= 2 c.cm.	Clear, reddish	3 c.cm.	Yellow, clear	
\mathbf{Frc}	om 2.50—2.5	5, 40 c.cm. of 1 % Indig	o carmine	in warm normal	
saline allowed to flow into peritoneal cavity.					
2.50 - 3.0	= 2 c.cm.	Clear, almost colourless	1.5 c.cm.	Blue at 2.57	
3.0-3.10	= 1.8 c.cm.	Clear and colourless	1.6 c.cm.	Deep blue	
3.10-3.30	0 = 3.4 c.cm.	Clear, bluish tinge to- wards end	4.6 c.cm.	Very dark blue	
3.30—4.5	= 10 c.cm.	Light blue	6·4 c.cm. dar	(some lost). Very k blue	
4.5 - 5.5	= 7.5 c.cm.	Same colour as last }	3.5 c.cm.	Very dark blue	

At 3.50 blood was drawn from femoral artery and centrifuged. The serum was bluish, about the same tint as the lymph between 3.30 and 4.5.

Dog killed at 5.15. 15 c.cm. of blue fluid (containing proteids) recovered from peritoneal cavity. Bile bright green. No staining of mesenteric glands. A gland in anterior mediastinum stained blue.

EXPERIMENT III.

May 13, 1893. Dog about 6 kilos. Y cannula in left pleural cavity.

At 12.55, 40 c.cm. of warm normal saline $(.75 ^{\circ})_{o}$ allowed to flow into pleural cavity.

At 4.25 dog killed. 25 c.cm. of fluid slightly blood-stained, recovered from pleural cavity. This was put in an ice chest for two days. At the end of that time the fluid was colourless and opalescent, all corpuscles having subsided. A delicate clot was floating in it. On boiling the clear fluid gave a large precipitate. It was found to contain $1.99 \, ^{\circ}/_{\circ}$ of total solids.

EXPERIMENT IV.

May 9, 1893. Dog about 6 kilos. Cannulae in thoracic duct and bladder. Y cannula in left pleura.

At 1.45 injected 30 c.cm. of 5 $^{0}/_{0}$ peptone solution in normal saline into pleural cavity.

Time	\mathbf{Lymph}	Urine	
1.45 - 2.45	= 3.8 c.cm. (no peptone)	2.8 c.cm. (no peptone)	
2.45 - 3.50	= 5.8 c.cm. (no peptone)	2 c.cm. (no peptone)	
3.50 - 4.45	= 2.6 c.cm. (no peptone)	3.4 c.cm., contains a fair amount o	f
		peptone	

Dog killed at 5.0 p.m. Pleural cavity contained 31 c.cm. of fluid, bloodstained. The fluid contained $3.5 \, {}^{0}/_{0}$ total solids, of which $1.3 \, {}^{0}/_{0}$ was peptone. A fair amount of coagulable proteid present.

EXPERIMENT V.

June 13, 1893. Dog about 7 kilos. Cannula in thoracic duct. Y cannula in left pleura.

Time	Lymph	
2.20 - 3.0 =	2.6 c.cm.	
3.0—3.10 =	2·3 c.cm	
3.10 - 3.25 =	3.5 c.cm.	At 3.10, 50 c.cm. of decoction
3.25-3.40 =	2.5 c.cm.	of mussels injected into
3.40-4.10 =	4.2 c.cm.	pleural cavity.
4.10-4.30 =	3 c.cm.	
4.30 - 5.40 =	10 c.cm.	

Dog killed at 5.45. The lymph kept on clotting in the cannula and hence the irregularity in the lymph flow.

40 c.cm. of blood-stained fluid recovered from the pleural cavity. The next day the fluid was found to have clotted. The serum contained a large amount of coagulable proteid.

(10 grams of dried mussels were boiled for 10 minutes with 100 c.cm. of normal saline, and filtered, and 50 c.cm. of the filtrate injected.)

EXPERIMENT VI.

June 20, 1893. Dog about 5 kilos. Cannulae in thoracic duct and bladder. Y cannula in left pleura.

 Time
 Lymph
 Urine

 11.40-11.55 = 2 drops
 $3\cdot 2$ c.cm. (no sugar)

 At 11.55 ran in 50 c.cm. of $10 \, {}^{0}/_{0}$ sol. of dextrose.

 $11.55-12.40 = 1\cdot 6$ c.cm.
 8 c.cm. (large amount of sugar)

 $12.40-1.40 = 1\cdot 8$ c.cm.
 6 c.cm. (,, ,, ,,))

Dog killed at 1.40. 96 c.cm. of fluid recovered from pleural cavities.

EXPERIMENT VII.

June 21, 1893. Dog about 5 kilos.

At 4.55, 50 c.cm. of a $10^{\circ}/_{\circ}$ solution of dextrose in normal saline injected into pleural cavity. Dog killed at 6.55. 88 c.cm. of fluid were recovered from the pleura. This contained $0.5^{\circ}/_{\circ}$ sugar, and a very slight amount of proteid.

EXPERIMENT VIII.

June 26, 1893. Dog about 6 kilos. Y cannula in left pleura.

At 11.10, 50 c.cm. of a $10 \, {}^{0}_{0}$ solution of NaCl run into pleura. Dog killed at 2.40. From pleural cavities were recovered 170 c.cm. of blood-stained fluid, containing a considerable amount of proteid.

EXPERIMENT IX.

June 28, 1893. Dog about 5 kilos. Y tube in left pleura.

At 3.30 p.m., 50 c.cm. of warm filtered distilled water run into pleural cavity. At 5.7 dog killed. P.-M. 30 c.cm. of fluid found in left pleural cavity, containing large amount of proteid ($\frac{1}{2}$ on boiling).

EXPERIMENT X.

July 27, 1893. Dog about 7 kilos. Cannula in thoracic duct and Y cannula in left pleural cavity.

Time Lymph
12.25—12.35 = 6.8 c.cm. Milky, a slight tinge of red
At 12.35 bled from femoral artery to 100 c.cm. This was defibrinated.
12.35—45' = 5.4 c.cm. Milky, slight tinge of red

At 12.45 ran 50 c.cm. of the defibrinated blood into the pleural cavity. $45' - 55' - 3\cdot 2$ c.cm. Same as last specimen

45 - 55 = 52 c.cm.	Same	\mathbf{as}	iast sp	ecimer
12.55 - 1.5 = 3.2 c.cm.	,,	;	,	,,
1.5 - 1.15 = 3.4 c.cm.	Very	slig	ghtly r	edder
15'-25' = 3.0 c.cm.	Same	as	before	
1.25-2.15 = 14 c.cm.	"	,,	"	
2.15 - 3.25 = 12 c.cm.	,,	;,	"	
3.25 - 4.5 = 4.4 c.cm.	,,	"	,,	

Dog killed at 4.5. P.-M. From left pleural cavity recovered 5 c.cm. of blood. Specimens of the blood were taken before it was put into the pleural cavity, and also of the blood taken out, and weighed and dried at 110° C.

Blood before injection contained $19.8 \,{}^{\rm o}/_{\rm o}$ solids.

After $3\frac{1}{2}$ hours in pleural cavity $18.7 \, ^{\circ}/_{\circ}$ solids.